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The effect of acute potassium depletion on muscle cell pH *in vitro*

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The effect of acute potassium depletion on muscle cell pH *in vitro*. It is generally assumed that potassium depletion alters muscle cell pH. *In vivo* techniques, usually show that muscle pH is decreased but *in vitro* experiments performed at an extracellular pH (pH_e) of 7.40 do not confirm this. To investigate this problem, intact rat diaphragms were incubated *in vitro* in a low potassium medium and results compared to those obtained in non-potassium depleted tissue. Varying pH_e was achieved by altering CO_2 tension (respiratory) or bath bicarbonate concentration (metabolic). Cell pH was measured simultaneously from distribution of the weak acid DMO (pH_{DMO}) or weak base, nicotine ($pH_{nicotine}$). At a pH_e of 7.40 both pH_{DMO} and $pH_{nicotine}$ were slightly but not significantly reduced ($P>0.10$) and pH heterogeneity was unchanged. In respiratory alkalosis, both pH_{DMO} and $pH_{nicotine}$ were significantly ($P<0.01$) reduced in potassium depleted tissue while in metabolic alkalosis only pH_{DMO} was so lowered. Much larger changes were found in both respiratory and metabolic acidosis. At all pH_e values less than 7.10, pH_{DMO} and $pH_{nicotine}$ were lower in potassium depleted tissue than in normal tissue ($P<0.001$). These data indicate that the discrepancy between *in vivo* and *in vitro* results is mainly apparent only at an external pH of 7.40. In alkalosis and acidosis muscle cell pH is decreased in potassium depletion.

Effet de la déplétion aigüe en potassium sur le pH de la cellule musculaire in vitro. Il est généralement admis que la déplétion en potassium modifie le pH cellulaire. Les techniques in vivo montrent habituellement que le pH musculaire est diminué mais les expériences in vitro réalisées à un pH extracellulaire (pH_e) de 7,40 ne confirment pas cela. Afin d'étudier ce problème des diaphragmes intacts de rats ont été incubés in vitro dans un milieu pauvre en potassium et les résultats comparés à ceux obtenus dans des tissus non déplétés en potassium. La variation de pH_e a été réalisée en modifiant la pression partielle de CO_2 (respiratoire) ou la concentration des bicarbonates (métabolique) du milieu. Le pH cellulaire a été mesuré simultanément à partir de la distribution de l'acide faible DMO (pH_{DMO}) ou de la base faible nicotine ($pH_{Nicotine}$). A un pH_e de 7,40 à la fois pH_{DMO} et $pH_{Nicotine}$ étaient diminués mais non significativement ($P<0,1$) et l'hétérogénéité du pH n'était pas modifiée. Dans l'alcalose respiratoire à la fois pH_{DMO} et $pH_{Nicotine}$ étaient significativement diminués ($P<0,01$) dans les tissus déplétés en potassium alors qu'en alcalose métabolique seul le pH_{DMO} était diminué. Des modifications beaucoup plus importantes ont été observées dans l'acidose tant respiratoire que métabolique. A toutes les valeurs de pH_e inférieures à 7,10, pH_{DMO} et $pH_{Nicotine}$ étaient inférieurs dans les tissus déplétés de potassium par rapport au tissu normal ($P<0,001$). Ces résultats indiquent que la discordance entre les résultats in vivo et in vitro apparait principalement à un pH externe de 7,40. En alcalose et en acidose le pH de la cellule musculaire est abaissé par la déplétion en potassium.

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For many years it has been assumed that the extracellular alkalosis of potassium depletion is accompanied by an intracellular acidosis [1]. Direct measurement of cell pH in this condition, however, has yielded conflicting results. *In vivo* experiments have generally shown that muscle cell pH is diminished in potassium depletion [2–4] but Miller, Tyson, and Relman [5], in an *in vitro* study using an intact rat diaphragm preparation, were not able to confirm this finding. At an external pH of 7.40 the pH of the potassium depleted tissue, calculated from distribution of the weak acid DMO (pH_{DMO}), did not differ significantly from that of non-potassium depleted muscle. They attributed this different result to better control of CO_2 tension which usually rises in the *in vivo* situation [6] but this contention has not been corroborated [7, 8].

Recently, Adler has demonstrated the presence of significant pH heterogeneity within rat diaphragm muscle [9]. It seemed possible that pH heterogeneity might differ in potassium depletion. To study this problem intact rat diaphragms were incubated at varying external acidities in a low potassium medium to induce a state of acute potassium depletion. Cell pH was measured simultaneously from the distribution of a weak acid, DMO (pH_{DMO}) and distribution of a weak base, nicotine ($pH_{nicotine}$). The arithmetic difference between the two calculations is a function of pH heterogeneity [9, 10]. The results suggest that potassium depletion does not affect pH heterogeneity and that

it does not significantly decrease cell pH at normal external pH values. However, at the alkaline external pH values normally seen in potassium depletion, the cell pH in potassium depleted tissue is lower than in non-depleted tissue. Also, the buffering capacity of diaphragm muscle is markedly decreased in the potassium depleted state both in respiratory and metabolic acidosis, indicating that potassium depleted muscle is able to defend itself less well than normal muscle against either of these two conditions.

Methods

Intact rat diaphragm were obtained from 75 to 90 g Sprague-Dawley rats and excess muscle tissue of the rib cage was dissected away. The diaphragms were then incubated simultaneously in two incubation chambers in a modified Krebs-Ringer bicarbonate solution as previously described [9]. Glucose, 100 mg/100 ml, was employed as substrate. The potassium concentration of the medium was found to be 0.3 mEq/liter after incubation despite no added potassium to the medium. The potassium source was the cut muscle fibers of the rib cage. In each experiment 12 diaphragms were incubated in each chamber for four hours at constant external conditions to assure attainment of a steady state. As previously described [9], inulin, 800 mg/100 ml, was added to the final medium as a measure of extracellular space and either 25 μCi of ^{14}C-DMO or 25 μCi of ^{14}C-nicotine was added to a chamber for the calculation of intracellular pH. Throughout the experiment 2 mM cold nicotine was present in each bath. Each analysis was carried out on a pool of two diaphragms and each experimental value, therefore, represents the mean of six analyses on the 12 diaphragms.

Respiratory experiments. Diaphragms were incubated as described but the pH of the bathing medium was altered by varying its CO_2 tension. Bicarbonate concentration was maintained constant in all experiments between the levels of 19.1 to 22 mEq/liter.

Metabolic experiments. In this group of experiments external pH was varied systematically by altering the bicarbonate concentration of the medium with reciprocal changes in chloride to maintain iso-osmolarity. Bath CO_2 tension was maintained constant at 34 to 40 mm Hg.

Analytical methods. Bath pH was measured using a Radiometer pH meter, CO_2 content of the medium was determined manometrically, and CO_2 tension was calculated from the pH and CO_2 content. Sodium and potassium of the medium and tissue were measured on an IL flame photometer. ^{14}C-compounds were counted in a Packard liquid scintillation counter and inulin was determined colorimetrically as previously described (9). Total tissue water was obtained by drying to constant weight. Intracellular pH was calculated from either ^{14}C-DMO (pH_{DMO}) or ^{14}C-nicotine ($pH_{nicotine}$) distributions using standard equations (2,10); pH heterogeneity is defined arbitrarily as pH_{DMO} minus $pH_{nicotine}$.

Results

Experiments performed at "normal" external pH. Table 1 compares the results of experiments performed at an external pH range of 7.37 to 7.44 and potassium concentration of 5.3 mEq/liter to those performed at a similar extracellular pH range of 7.39 to 7.44 in a medium containing 0.3 mEq/liter potassium. There is a decrease in both pH_{DMO} and $pH_{nicotine}$ in the potassium depleted tissue but the differences are not statistically significant. In addition, there is no significant change in pH heterogeneity. The decrease in tissue potassium concentration is essentially the same as the rise in sodium concentration. Thus, the sum of the intracellular sodium and potassium concentrations in normal tissue is 178 mEq/liter compared to 179 mEq/liter in the potassium depleted tissue. This agrees with other results reported *in vitro* [11], though it is at variance with the chronic potassium depletion data reported by Cooke et al [1].

Experiments performed at varying degrees of CO_2 tension (respiratory). The results of these experiments are shown in Table 2. In all experiments tissue potassium concentration was significantly lowered compared to previous work employing non-potassium depleted tissue [9]. With increasing CO_2 tension and decreasing extracellular pH both pH_{DMO} and pH nicotine progressively fell. Fig. 1 compares the changes of cell pH in the potassium depleted tissue with previously published data obtained from non-potassium depleted diaphragms [9]. At all alkaline extracellular

Table 1. Effect of potassium depletion on tissue water, electrolytes and pH at an external pH of approximately 7.40[a]

Group	Total H_2O %	Ext. Space %	$[Na^+]$ *mEq/liter*	$[K^+]$ *ICW*	$[K^+]$ *mEq/kg DW*	pH_{DMO}	$pH_{Nicotine}$	$pH_{Heterogeneity}$
Non-potassium depleted[b] ($N=35$)	74.5±1.3	24.4±2.5	24±6	154±13	341±16	7.17±0.06	6.69±0.07	0.48
Potassium depleted ($N=18$)	74.0±2.4	19.4±3.6	86±10	93±12	214±28	7.12±0.04	6.66±0.03	0.46
P[c]	>0.2	<0.01	<0.001	<0.001	<0.001	>0.10	>0.30	>0.50

[a] Each number represents the mean ± SD.
[b] Obtained from this laboratory's previously reported data [9].
[c] Student t test comparing the two groups.

Table 2. Effect of progressive changes in CO_2 tension on tissue electrolytes and pH in potassium depleted tissue[a]

Medium		Tissue					
P_{CO_2} *mm Hg*	pH	$[Na^+]^b$ *mEq/liter ICW*	$[K^+]^b$ *mEq/liter ICW*	$[K^+]^b$ *mEq/kg DW*	$pH_{DMO}{}^b$	$pH_{Nicotine}{}^b$	$pH_{Heterogeneity}$
10	7.96	78 ± 8	93 ± 7	229 ± 17	7.46 ± 0.05	6.91 ± 0.02	0.55
19	7.70				7.23 ± 0.04	6.78 ± 0.03	0.45
24	7.60	61 ± 7	93 ± 11	215 ± 12	7.15 ± 0.03	6.76 ± 0.04	0.39
31	7.42	82 ± 10	90 ± 8	212 ± 12	7.12 ± 0.04	6.68 ± 0.03	0.44
34	7.44	79 ± 7	98 ± 5	223 ± 8	7.11 ± 0.04	6.63 ± 0.03	0.48
37	7.39	97 ± 9	91 ± 15	208 ± 28	7.15 ± 0.03	6.68 ± 0.02	0.47
62	7.17	67 ± 9	86 ± 3	193 ± 11	6.93 ± 0.02	6.65 ± 0.03	0.28
83	7.04	76 ± 7	88 ± 9	183 ± 14	6.74 ± 0.05	6.55 ± 0.02	0.19
145	6.82	80 ± 8	95 ± 5	176 ± 16	6.62 ± 0.05	6.36 ± 0.03	0.26

[a] Each number represents the mean of six determinations.
[b] Mean ± SD

pH values both pH_{DMO} and $pH_{nicotine}$ are lower in the potassium depleted tissue leaving intracellular pH values closer to those found when extracellular pH is 7.40. A similar change, but of greater magnitude, is shown at acid extracellular pH values. Thus, at an external pH of 6.82 pH_{DMO} is 0.19 pH units and $pH_{nicotine}$ 0.32 pH units lower than that found in non-potassium depleted tissue at the same external pH [9]. These differences are significant ($P<0.001$). In addition, the remarkable resistance of $pH_{nicotine}$ to change with increasing CO_2 tension which was demonstrated in the non-potassium depleted tissue is virtually eliminated in the potassium depleted state. Since the cell is freely permeable to carbon dioxide, the lower cell pH in potassium depleted tissue compared to the non-depleted tissue as the CO_2 tension is progressively raised, suggests that buffering capacity in potassium depletion is decreased.

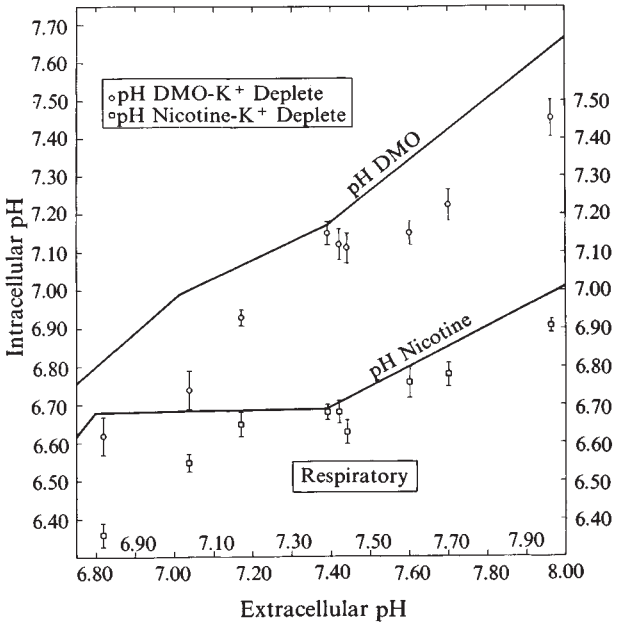

Fig. 1. *Effect of potassium depletion on cell pH compared to that of non-potassium depleted tissue as a function of varying extracellular pH by alterations in bath CO_2 tension at a constant bicarbonate concentration.* The solid lines indicate the relationship previously reported for non-potassium depleted tissue at the same fixed bicarbonate concentration. Points are the values obtained in the present study with potassium depleted tissue and each is the mean ± SD of six analyses.

Experiments performed at varying external bicarbonate concentrations (metabolic). The effect on cell pH of changing bath bicarbonate concentration while bath CO_2 tension is held constant is shown in Table 3. As was true in the respiratory experiments, progressive lowering of extracellular pH induced by decreasing external bicarbonate concentration results in progressive decreases in both pH_{DMO} and $pH_{ntcotine}$. In all experiments tissue potassium concentration was significantly less than in previous experiments carried out under the same external conditions in the presence of 5.3 mEq/liter potassium in the bathing medium [9]. At an alkaline extracellular pH induced by increasing external bicarbonate concentration (Table 3), pH heterogeneity is reduced compared to the values seen at similar external pH values caused by decreases in CO_2 tension (Table 2). The reason for this difference in heterogeneity during metabolic alkalosis is shown in Fig. 2 where it is apparent that in the potassium depleted state pH_{DMO} is more decreased than $pH_{nicotine}$ when compared to the results found in non-potassium depleted muscle.

In metabolic acidosis cell pH in potassium depletion responds in a fashion similar to that seen in respiratory acidosis (Fig. 1). Thus, at an external pH of 6.84, pH_{DMO} is 0.26 pH units and $pH_{nicotine}$ is 0.17 pH units lower than that expected when tissue potassium is normal (9). These results are significant at the 0.001 level. As it cannot be shown that the tissue is freely permeable to hydrogen, hydroxyl, or bicarbonate ions this relative decrease in cell pH may not be due to a decrease in buffering capacity as is suggested by the results in respiratory acidosis. Nevertheless, it is obvious that in either respiratory or metabolic acidosis muscle cell pH is lowered to a greater extent when the muscle is depleted of potassium.

Table 3. Effect of progressive changes in bicarbonate concentration on tissue electrolytes and cell pH in potassium depleted tissue[a]

Medium		Tissue					
$[HCO_3^-]$ *mEq/liter*	pH	$[Na^+]$[b]	$[K^+]$[b] *mEq/liter ICW*	$[K^+]$[b] *mEq/kg DW*	pH_{DMO}[b]	$pH_{Nicotine}$[b]	$pH_{Heterogeneity}$
64.6	7.90	56± 4	91± 4	226±12	7.27±0.03	7.03±0.08	0.24
30.0	7.62	64±15	93± 9	203±17	7.15±0.08	6.87±0.03	0.28
22.0	7.44	79± 7	98± 5	223± 8	7.11±0.04	6.63±0.03	0.48
21.7	7.39	97± 9	91±15	208±28	7.15±0.03	6.68±0.02	0.47
19.1	7.42	82±10	90± 8	212±12	7.12±0.04	6.68±0.03	0.44
12.1	7.18	54± 7	97± 8	212±14	6.94±0.03	6.66±0.02	0.28
8.7	7.02	58±10	89±22	178±29	6.82±0.03	6.48±0.02	0.34
8.7	7.02	48± 7	87±11	181±16	6.78±0.03	6.51±0.05	0.27
6.4	6.84	61± 8	91± 3	193±11	6.67±0.02	6.34±0.03	0.33

[a] Each number represents the mean of six determinations.
[b] Mean ± SD.

Discussion

Since most enzymes exhibit pH optima [12], cell pH must be a potent regulator of cellular metabolism. Yet, in potassium depletion, a state increasingly found in clinical medicine with the advent of modern diuretics, the relatively infrequent muscle abnormalities seen appear to be related more to changes in transmembrane potential [13] than to metabolic alterations which might be associated with changes in intracellular acidity. Though the present study suggests that pH heterogeneity is unaltered, it does help explain the apparent lack of metabolic changes occurring in muscle in potassium depletion. Usually, in this state, extracellular pH is raised with pH values ranging between 7.45 and 7.60 [14]. In rat diaphragm muscle, over this same external pH range, pH_{DMO}, in either metabolic or respiratory experiments lies close to the cell pH found at an external pH of 7.40 in the absence of potassium depletion. Although pH nicotine does increase, especially in metabolic alkalosis, the overall effect on cell pH seems small. In addition, CO_2 tension may be elevated in potassium depletion and this might bring cell pH towards normal [6, 10]. Miller, Tyson, and Relman [5] examined the effect of high P_{CO_2} on diaphragm muscle *in vitro* at a fully compensated external pH of 7.39. In agreement with the results reported in this paper they found little difference in pH_{DMO} between potassium depleted and non-depleted tissue at this external pH. It is true that they did not examine pH heterogeneity but it seems probable that muscle cell pH is not significantly altered in uncomplicated potassium depletion.

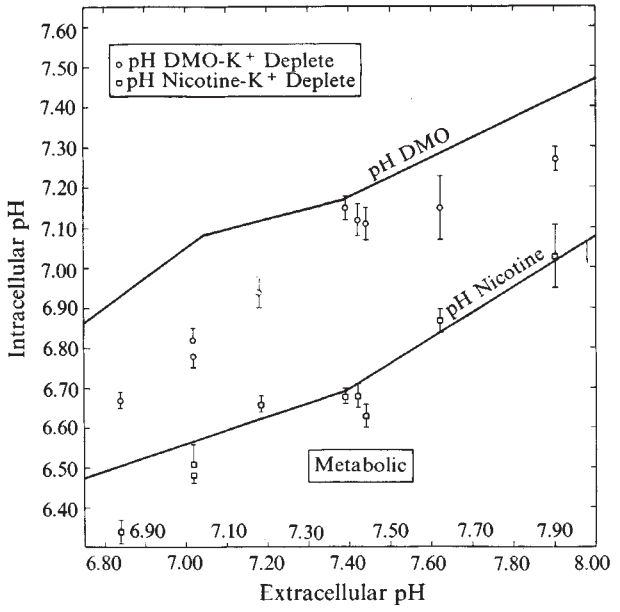

Fig. 2. *Effect of potassium depletion on cell pH compared to that of non-potassium depleted tissue at varying extracellular pH values achieved by altering the external bicarbonate concentration at a constant bath CO_2 tension of 34 to 40 mm Hg.* The solid lines indicate the relationship previously reported for non-potassium depleted tissue at a fixed CO_2 tension of 34 to 41 mm Hg. Points are the values obtained in the present study with potassium depleted tissue and each represents the mean ± SD of six analyses.

The present experiments help to resolve the apparent contradiction between previous *in vitro* results and data obtained *in vivo* [2–4]. The latter experiments with potassium depletion almost always have shown a decrease in muscle cell pH. Usually, however, the CO_2 tension was elevated, a situation which should lead to a lower cell pH when muscle tissue is potassium depleted [7, 8]. Indeed, in the *in vivo* experiments in which a CO_2 titration curve in potassium depletion was compared to normal there was a decrease in buffer capacity in the former state [7, 8]. However, as the CO_2 tension approached 40 mm Hg and external pH approached 7.40, the bicarbonate concentration in the depleted and nondepleted tissue became similar – a result consistent with little change in cell pH in potassium depletion at "normal" CO_2 tensions. This explains why Miller, Tyson, and Relman [5] *in vitro*, did not demonstrate a change in cell pH in diaphragm muscle in acute potassium depletion since their experiments were performed at a single extracellular pH value of 7.40. It is precisely under these external conditions that muscle cell pH is least

affected by potassium depletion. Both the present data and the *in vivo* data demonstrate decreased carbon dioxide buffering capacity in muscle depleted of potassium [7, 8]. The present experiments also show that in metabolic acidosis intracellular pH in the potassium depleted muscle cell is affected more than in non-depleted tissue.

Muscle cell pH lies outside of thermodynamic equilibrium, being an entire pH unit higher than predicted [9, 10, 15, 16]. This implies that there are active mechanisms which regulate muscle cell pH. Since the cell is significantly more acidic in the potassium depleted state in both metabolic and respiratory acidosis, it appears that potassium depletion must interfere with the cell's ability to actively extrude hydrogen ions and/or transport hydroxyl or bicarbonate ions into its interior. It is tempting to extrapolate from these *in vitro* findings and speculate on the effect of superimposed acidosis in the potassium depleted state but further work should be done before accepting the relevance of these results to *in vivo* states.

It has been stated that the simultaneous increase or decrease in the chemical potential of hydrogen and potassium ions in intracellular water with respect to their chemical potential in extracellular water is due to a common factor [17]. The present experiments indicate that this is probably untrue since at an external pH of 7.40 the intracellular to extracellular potassium concentration ratio is approximately 30 in normal tissue while it is 300 in the potassium depleted state – an order of magnitude difference. Yet, cell pH is not significantly altered so that intracellular to extracellular hydrogen ion ratios appear to be unaffected by potassium depletion. Sanslone and Muntwyler [18] have also shown that muscle cell pH in potassium depleted muscle can remain the same despite large changes in muscle potassium. It seems, therefore, that hydrogen ion movement is not necessarily dependent on the transcellular potassium gradient. Kim and Brown [19], and Makoff, DaSilva and Rosenbaum [20] have recently come to similar conclusions for potassium distribution in muscle by showing that potassium movement could not be well correlated with the intracellular-extracellular hydrogen ion ratio. The exact cause of the intracellular pH changes in potassium depletion, therefore, is still unknown.

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